Supplementary Material to:
Theory of lethal mutagenesis for viruses

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We derive the extinction threshold and show that gene frequency evolution is independent of population growth/decline. We assume that the population is large enough that gene frequency evolution obeys average fitnesses and mutation rates. We use the symbols as defined in Supplementary Table 1. Note that the w_i are fixed, but $\overline{w}$ is not. We set an upper bound $S_{\max} < 1$ on the survival function $S(N)$, i.e., $0 \leq S(N) \leq S_{\max}$. This upper bound expresses the assumption that offspring survival is never 100%.

Supplementary Table 1. Symbols used in analytical derivations.

symbol	definition
n_i	absolute number of mature virions carrying i mutations
N	total population size $N = \sum_i n_i$
p_i	relative frequency of mature virions carrying i mutations ($p_i = n_i/N$)
$m_{j,i}$	proportion of offspring with j mutations from a parent with i mutations ($i < j$)
w_i	relative fitness (offspring number) of a mature virions with i mutations ($w_0 = 1$)
b	"burst size", absolute number of offspring produced by wild type or best virus
bw_i	absolute number of offspring of a parent with i mutations
$S(N)$	offspring survival to mature virus; strictly declining with N, independent of genotype
$\overline{w}$	mean relative fitness ($\overline{w} = \sum_i \sum_j p_j w_j m_{j,i}$)

Extinction threshold. With the definitions of Supplementary Table 1 and using primes to denote values in subsequent generations, the dynamics of population numbers is

$$n_i' = bS(N) \sum_j n_j w_j m_{j,i} \,, \tag{1}$$

$$N' = \sum_i n_i' = \overline{w} N b S(N) \,. \tag{2}$$

Gene frequency changes are given by

$$p_i' = \frac{n_i'}{N'} = \frac{\sum_j p_j w_j m_{j,i}}{\overline{w}} \,, \tag{3}$$

hence independent of population size. A similar result holds in the continuous-time version of these equations (4).

For population decline, $N' < N$, which is simply

$$N' = \overline{w}' N b S(N) < N \tag{4}$$

and thus

$$\overline{w}' b S(N) < 1 \,. \tag{5}$$

For this inequality to hold indefinitely, we use equilibrium mean fitness and $S(N) = S_{\max}$. Writing $R_{\max} = b S_{\max}$ generates our threshold.

Mutation number in generations 1 and 2 before and after selection. We next derive the results underlying Table 1 in the main text, especially the effect of mutation and selection in the first generation.

We assume that a largely mutation-free template is used to infect cells and will be subjected to mutagenesis in those cells. Such a template may be obtained from a DNA virus at the normal mutation rate or from an RNA virus transcribed from a DNA template (1). The infection process could be a normal one (virions infecting cells) or done by transfecting RNA/DNA into cells. To the extent that these genomes are all the same sequence, fitness effects of mutations are irrelevant at this point. As the next step in this process, those infections produce progeny virus with mutation rate U per genome. Ideally, the number of virions produced with different genomic mutations depends only on the Poisson probabilities and not on any selective effects of those mutations, because those genomes were packaged without being expressed (an obvious violation of known biology but perhaps not seriously affecting observed numbers). Upon viral release from this first round of infection, and before subsequent infection, all mutations behave as if neutral, and the average observed number will be U per genome. This result creates the first line in Table 1 in the main text (generation 1, after mutation and before selection). One would observe this mutation count in the genomes of the individual virions released, before they go on to infect.

Allowing those virions to infect new cells and produce progeny now exposes 1st-generation mutations to selection. Genomes with lethal mutations will leave no progeny, and those with only non-lethal mutations will produce correspondingly fewer progeny than nonmutated genomes. The mean fitness is the expected number of 2nd-generation progeny, and we will calculate this quantity below. When those 2nd-generation virions are released, they will not only carry mutations of the first generation (appropriately winnowed by selection), but they will also carry a second generation of mutations. Thus, in the second generation of mutation before selection, the total mutation count is now U plus the average that survived selection from the first generation in the parents. Thus the third line in the table is simply U plus the second line.

We now derive the mean fitness and mean number of mutations after 1 generation of mutation and selection. Mean fitness in the first generation is merely the sum over i of the product of the probability of having i mutations times the fitness effect of i mutations:

$$\overline{w}(1) = \sum_{i=0}^{\infty} w_i e^{-U_d} \frac{U_d^i}{i!} \,. \tag{6}$$

The average number of mutations in the first generation $\overline{m}(1)$ is then

$$\overline{m}(1) = U_n + \sum_{i=0}^{\infty} i w_i e^{-U_d} \frac{U_d^i}{i!} \Big/ \overline{w}(1) \,. \tag{7}$$

In the following, we develop formulae 6 and 7 for each of our specific models.

Multiplicative fitness. The first-generation effect of mutation on fitness (after selection) is found as

$$\overline{w}(1) = \sum_{i=0}^{\infty} (1-s)^i e^{-U_d} \frac{U_d^i}{i!} = e^{-sU_d} \,. \tag{8}$$

The average number of mutations after this selection is

$$\overline{m}(1) = U_n + \sum_{i=1}^{\infty} e^{-U_d} i (1-s)^i \frac{U_d^i}{i!} \Big/ \overline{w}(1) = U - sU_d \,. \tag{9}$$

The average number of non-neutral mutations at equilibrium was given in Ref. (2).

Eigen model. Starting with a mutation-free genotype, any genotype that acquires one or more mutations drops in fitness to $1-s$, and only the zero class retains fitness 1. Thus, mean fitness after one generation of mutation in the Eigen model drops to

$$\overline{w}(1) = e^{-U_d} + (1-s)(1 - e^{-U_d}) = 1 - s + s\, e^{-U_d} \,. \tag{10}$$

The average number of mutations after selection is

$$\begin{aligned}
\overline{m}(1) &= U_n + (1-s) \sum_{i=1}^{\infty} i e^{-U_d} \frac{U_d^i}{i!} \Big/ \overline{w}(1) \\
&= U - \frac{U_d s\, e^{-U_d}}{1 - s + s\, e^{-U_d}} \,.
\end{aligned} \tag{11}$$

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The average number of mutations at equilibrium was given in Ref. (3).

Truncation selection. Starting with no mutations, the mean fitness after one generation of mutation in the truncation model is simply the fraction of genotypes that received k or fewer non-neutral mutations:

$$\overline{w}(1) = \sum_{i=0}^{i=k} e^{-U_d} \frac{U_d^i}{i!} \, . \tag{12}$$

The average number of mutations is

$$\overline{m}(1) = U_n + \sum_{i=1}^{k} i e^{-U_d} \frac{U_d^i}{i!} \Big/ \overline{w}(1) \, . \tag{13}$$

For $k = 1$, this expression becomes

$$\overline{m}(1) = U - \frac{U_d^2}{1 + U_d} \, . \tag{14}$$

Our last goal is to derive the number of mutations at equilibrium. Letting x_i be the frequency of a genome with i non-neutral mutations measured after mutation and before selection, we may write the change in frequency of the genotype with 0 mutations as

$$x_0' = x_0 e^{-U_d} / \overline{w} \, , \tag{15}$$

which means that in equilibrium, we have either $\overline{w} = e^{-U_d}$ or $x_0 = 0$. If $x_0 = 0$, then an expression equivalent to equation 15 holds for x_1, $x_1' = x_1 e^{-U_d} / \overline{w}$, and we can repeat the argument until we reach x_k. For x_k, a non-zero equilibrium is $x_k = e^{-U_d}$. We now demonstrate that this solution is unique.

Assume that there is a non-zero x_j for $j < k$, with $x_i = 0$ for $i < j$. Then, we have

$$x_j' = x_j e^{-U_d} / \overline{w} \, , \tag{16}$$

from which we again obtain $\overline{w} = e^{-U_d}$. Then, x_k changes as

$$
\begin{aligned}
x_k' & = x_k e^{-U_d} / \overline{w} + \sum_{i=j}^{k-1} x_i e^{-U_d} \frac{U_d^{i-j+1}}{(i-j+1)!} \Big/ \overline{w} \\
& = x_k + \sum_{i=j}^{k-1} x_i \frac{U_d^{i-j+1}}{(i-j+1)!} \, ,
\end{aligned}
\tag{17}
$$

which implies $\sum_{i=j}^{k-1} x_i \frac{U_d^{i-j+1}}{(i-j+1)!} = 0$. Since all x_j are non-negative, this condition implies that x_j, x_{j+1}, ..., x_{k-1} are all equal to zero. Thus, there can be no $j < k$ for which $x_j > 0$, which proves that our solution is unique.

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